

Dear Recommender,

We are pleased to submit a revised version of the manuscript entitled 'Sub-lethal insecticide exposure affects host biting efficiency of Kdr-resistant Anopheles gambiae ' authored by Malal M. Diop et al. to be considered for recommendation in PCI Entomology.

We are very grateful to the referees for their meticulous and relevant comments on the manuscript. You will find below our response to the suggestion of the second referee.

Look forward to hearing from you at your earliest convenience,

Best regards,

Nicolas Moiroux on behalf of the authors

Reviewed by anonymous reviewer, 2020-02-27 15:21

I had evaluated the previous version of this article. I would like to thank the authors for taking into account my first remarks and especially the one concerning the KDR phenotype. I remain unsatisfied with the authors' discussion of the implication of these results in terms of their repercussions on the evolution of resistance in populations of this mosquito species. To facilitate the reading of the results of the article, I have tried to make a table summarizing the observed results. Here is the result of this attempt:

Tables.

Taking the time to make this assessment, it appears - but I may be mistaken - that the authors did not compare the value of several life history traits (eg probing event, probing duration...) between genotypes following the exposure of nets impregnated with permethrin and/or deltamethrin. These are all comparisons where I put a "?" in the table.

These comparisons could be made and even seem to me crucial to understand the evolution of resistance because this evolution depends not on the absolute value of these traits but rather on the relative value of the three genotypes in a given environment.

Actually, the authors did compare the values of the different life history traits in the absence of treatment. They also compared the blood feeding success of RR, RS and SS following exposure of mosquitoes to the two types of treated nets. Why not compare the values of other life history traits of the three genotypes (SS s RS, SS vs RR and RS vs RR) after exposure to nets treated with permethrin and deltamethrin? I'm sorry I didn't notice the absence of these comparisons when I first read it.

The presentation of a synthesis of the comparisons as above (or in an improved form) might be useful for the readers and for strengthens their discussion.

We thank again the reviewer for its suggestions. The reviewer made a helpful work for summarizing our results that pointed out that some comparisons (among genotypes for several parameters) were not presented. Although these supplementary comparisons do not change our

discussion and conclusion, we agree that there should be presented. It is now done in the revised version of the manuscript in which we added a paragraph in the Results section:

“When comparing the biting behavior among genotypes after insecticide exposure, we found that prediuresis duration of RS mosquitoes was shorter than that of SS mosquitoes after permethrin exposure ($HR_{RS-SS} = 3.82 [1.15, 12.7]$, supplementary Table 8). Moreover, prediuresis duration of RR mosquitoes was shorter than that of SS after deltamethrin exposure ($HR_{RR-SS}=3.41 [1.13, 10.29]$, supplementary Table 8). For all other parameters, we were not able to evidence any differences among genotypes (Supplementary Tables 1, 2, 7 and 8).”

We also added/updated several supplementary tables resulting from new analyses in the updated version of our R code (available online). We updated the Tables produced by the reviewer (see Table 1 and 2, below).

The reviewer wrote: *« These comparisons [...] seem to me crucial to understand the evolution of resistance because this evolution depends not on the absolute value of these traits but rather on the relative value of the three genotypes in a given environment. »*

We fully agree with him. This is actually the reason why we did not investigate more deeply on Kdr gene evolution, while slightly discussed in the second paragraph of the discussion. Our aim was to focus on contrasting impacts of behavioral modulations on the genotype fitness, but we did not want to decipher with different Kdr evolution scenarios under different selective pressure in fluctuating environments. Indeed, we believe that there is a risk to dilute our results and make the paper too long, too speculative and more confusing for the readers. Nevertheless, as for malaria transmission, we believe this is a major perspective supported by our data to model kdr evolution in fluctuating environments. We therefore modify the last sentence of the paper accordingly.

“All these interactions should then be used as variables to include host-seeking behavioural modulation by kdr resistance in models of resistance evolution and P. falciparum transmission to better understand and/or predict the efficacy of vector control strategies (74).”

We are not sure to have the resources to do such a work in the near future in our lab. Making our codes and data available allows scientific community interested in such modeling study to do it.

Finally, we removed the following sentence from the discussion section as it was too speculative: *“The whole picture suggests that kdr mutation may increase vectorial capacity of An. gambiae populations in areas where permethrin ITNs are implemented.”*

Table 1: Summary Result of treatment comparisons for blood feeding success and biting behavior parameters

Measured parameters	Genotype	Permethrin treated net		Deltamethrin treated net	
		effect on the measured parameter	expected effect on Kdr frequency (vs. UTN)*	effect on the measured parameter	expected effect on Kdr frequency (vs. UTN)*
Number of probing attempts	SS	→	→	→	→
	RS	→		→	
	RR	→		→	
Probing duration	SS	→	→	→	→
	RS	→		→	
	RR	→		→	
Blood feeding success	SS	↓	↑	→	↓
	RS	→		↓	
	RR	↑		↓	
Blood meal size	SS	↓	↑ ↓	↓	↑ ↓
	RS	↓		↓	
	RR	↓		↓	
Feeding duration	SS	↓	↑ ↓	↓	↑ ↓
	RS	↓		↓	
	RR	↓		↓	
Prediuresis duration	SS	→	↑ ↓	↓	↑ ↓
	RS	↓		↓	
	RR	↓		↓	
Legend for measured effects		Legend for expected effect			
↑	significantly higher	↑	increase		
↓	significantly lower	↓	decrease		
→	no significant differences	↑ ↓	increase or decrease		
*all other parameters being equals		→	no effect		

Table 2: Summary Result of genotype comparisons for blood feeding success and biting behavior parameters

Measured parameters	treatment	Comparison			expected dynamic of Kdr frequency
		RR vs SS	RR vs RS	RS vs SS	
Number of probing attempts	UTN	→	→	→	→
	PER	→	→	→	→
	DEL	→	→	→	→
Probing duration	UTN	→	→	→	→
	PER	→	→	→	→
	DEL	→	→	→	→
Blood feeding success	UTN	→	→	→	→
	PER	↑	↑	→	↑
	DEL	→	→	→	→
Blood meal size	UTN	→	↓	→	↑ ↓
	PER	→	→	→	→
	DEL	→	→	→	→
Feeding duration	UTN	↓	↓	→	↑ ↓
	PER	→	→	→	→
	DEL	→	→	→	→
Prediuresis duration	UTN	↓	↓	→	↑ ↓
	PER	→	→	↓	↑ ↓
	DEL	↓	→	→	↑ ↓
Legend for measured effects		Legend for expected effect			
↑	significantly higher	↑	increase		
↓	significantly lower	↓	decrease		
→	no significant differences	↑ ↓	increase or decrease		
*all other parameters being equals		→	no effect		

